REMARKS

In compliance with 37 C.F.R. § 1.821 through § 1.825, applicants have amended the specification to add Sequence Identifiers. No new matter has been added by this amendment. Applicants respectfully request entry of the present amendment.

Attached hereto is a marked up version of the changes made to the specification by the current amendment with additions underlined and deletions bracketed. The attached pages are captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**".

CONCLUSION

In the unlikely event that the transmittal letter is separated from this document and/or the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 220002058901. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Dated: March 1, 2002 By: _____

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

METHODS AND COMPOSITIONS FOR IDENTIFYING VARIATIONS IN HUMAN α_{1B}- AND β_2- ADRENERGIC RECEPTOR GENES

In the specification:

The paragraph on page 10, containing line 1, has been amended as follows:

(Amended)

Figure 5 **(SEQ ID NO:11)** depicts the sequence profile of fragment A of the human α_{1B}-AR gene. The amplified fragments were sequenced with the amplification,primer SEQ ID NO: 1 using Applied Biosystems' DNA sequencer. Each peak indicates a different nucleotide. In some cases "ambiguous reads" (N) occur as the result of duplicate bases (heterozygosity) or reading ambiguities, which can generally be corrected afterwards.

The paragraph on page 10, containing line 7, has been amended as follows:

(Amended)

Figure 6 **(SEQ ID NO:12)** depicts the sequence profile of fragment A of the human β_2-AR gene. The amplified fragments were sequenced with the amplification primer SEQ ID NO:6 using Applied Biosystems' DNA sequencer. Each peak indicates a different nucleotide. In some cases "ambiguous reads" (N) occur as the result of duplicate bases (heterozygosity) or reading ambiguities, which can generally be corrected afterwards.

The paragraph on page 27, containing line 30, has been amended as follows:

(Amended)

In a preferred embodiment, the method is used for diagnosing nocturnal asthma based on glycine 16 polymorphism in ϑ_2-AR gene. In another embodiment, the method is used for a diagnosis of essential hypertension based on the same genetic polymorphism. While assaying for glycine 16 polymorphism, genomic DNA of the test subject can be obtained from a blood sample. The N-terminal fragment A (as shown in Figure 2) that encompasses sequences

encoding the residue glycine 16 can be amplified using primers SEQ ID NOS:5 and 6, or derivatives thereof. Direct sequencing of the amplified products using the same primers employed in the amplification procedure may be performed to detect the single base change (adenosine to guanosine) at position 46 (numbering from the start codon) that results in amino acid substitution of arginine with glycine. Homozygosity of glycine 16 indicates the presence or a predisposition to nocturnal asthma and/or essential hypertension. Glycine 16 homozygotes will be apparent by comparing the sequence peaks from the subject sample to those of the controls (for an example of the sequence output from an automated DNA sequencer, see Figure 5 **(SEQ ID NO:11)** or 6 **(SEQ ID NO:12)**). A single peak corresponding to 27 nucleotide guanosine at position 46 is indicative of glycine 16 homozygosity. Two overlapping peaks, each representing guanosine or adenosine respectively at position 46, suggest heterozygosity of glycine 16. Finally, a single peak representing adenosine shows that the subject is homozygous in arginine 16.

The paragraph on page 31, containing line 24, has been amended as follows:

(Amended)

Example 5

Sequence Analysis

The PCR products of expected sizes were cut from the gel and the DNA was purified using QIAquick Gel Extraction Kit. The extracted DNA was resuspended in Tris-EDTA buffer (10mM Tris-Cl, 1mM EDTA, pH 8. 0) and concentrated using a Centricon Concentrator (Amicon). The purified gene fragments were then sequenced by an automated DNA sequencer (Applied Biosystems, model 377) using one or more of the same primers employed in PCR. The upstream primer SEQ ID NO: 1 and the downstream primer SEQ ID NO: 6 were used for sequencing the amplified products, fragment A of the human I_{1B}-adrenergic receptor gene and fragment A of ϑ_2-adrenergic receptor gene, respectively. As shown in Figure 5 **(SEQ ID NO:11)** and 6 **(SEQ ID NO:12)**, each sequencing read approximately 550 bases. Other primers

described herein including primer SEQ ID NOS: 1, 2, 3, 5, and 7 can also be used for direct sequencing with high reliability. By use of an automated sequencer and sequencing PCR products from in excess of 15 different subjects, we obtained consistent results in accordance with the published coding sequences of the human ϑ_2- and exon 1 of the human I_{1B}-adrenergic receptor. Repeated sequencing of PCR products of the same individuals revealed a 100% reliability of our PCR methods without requirement for repeat isolation of PCR fragments. Occasionally occurring "ambiguous reads", which are the result of a reading error of the automated sequencer, can generally be corrected afterwards without re-isolating and sequencing the PCR fragments (Figures 5 and 6).